

Scanning Electron Microscopic Observations on the Blood Cells of Common Carp (*Cyprinus carpio*) and Catfish (*Heteropneustes fossilis*) Under Piscicide Toxicity

SUMIT HOMECHAUDHURI
SAMIR BANERJEE

*Aquaculture Research Unit
Department of Zoology
University of Calcutta
35 Ballygunge Circular Road
Calcutta 700 019
India*

Abstract - Microtopographical studies on the blood cells of an air-breathing catfish, (*Heteropneustes fossilis*), and a non-airbreathing fish, common carp (*Cyprinus carpio*), under the toxic stress of a plant-derivative piscicide, have been made by SEM. Saponin, the active component of the toxicant, Mahua oil cake, is known to have strong hemolytic action. This note describes changes in the surface structure of the erythrocytes on treatment. The resultant physiological implications have been analyzed to judge the response of fish to this toxicity.

Piscicides are generally used in specified doses during prestocking pond management to eradicate predatory and weed fishes. Toxicants of plant origin have been preferred. In India, Mahua oil cake (MOC), a derivative from the plant *Bassia latifolia* (Family Sapotaceae), is extensively used as a fish toxicant. It is further useful as an organic manure. MOC contains 4.6% saponin, the hemolytic ingredient. Until recently, experimental efforts to evaluate the toxicity of different piscicides had been confined to studies on mortality rate. Bhatia (1970) was probably the first to assess the toxicity of MOC to freshwater fishes and indicated its usefulness in fisheries management. The preliminary assessment of the toxicity was worked out by other workers (Lakshmanan 1983; Chakroborty et al. 1987) both in laboratory and under field conditions. In our previous experiment (Homechaudhuri et al. 1986), the hemotoxic action of MOC on fish was determined using light microscopy. The present experiment records the changes in microtopographical features of the blood cells by scanning electron microscopy (SEM) to confirm the earlier observations.

Live specimens of catfish or singhi fish (*Heteropneustes fossilis*) and common carp (*Cyprinus carpio*) were obtained from a local fish farm. Healthy and disease-free fish were isolated and acclimated to laboratory conditions for 72 hours prior to toxicant exposure in 50-l plastic pools of pond water. The body lengths of *C. carpio* and *H. fossilis* were 17.5-19.0 and 16.5-18.5 cm, respectively. For each species, 12 specimens were used for control and 9 for the treatment. All the fish used were female for uniformity in results.

A dose of 250 mg.l⁻¹ was recommended by the Central Inland Fisheries Research Institute, India (CIFRI 1968) for total eradication of weed fishes during pisciculture. Powdered MOC of the same dose was applied to the fish until they showed signs of distress. Some of the hydrological parameters measured before the experiment were temperature (28.4°C), pH (8.25), dissolved oxygen (8.40 ppm), total hardness (220 ppm) and free dissolved carbon dioxide in traces.

Free-flowing blood was collected by severing the caudal peduncle of unanesthetized fish. Drawn blood (20 µl from each specimen) was fixed in freshly prepared 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature for two hours. The samples were centrifuged for a few minutes at 500-1,000 rpm to remove excess glutaraldehyde and plasma. The buffycoat layer was centrifuged, then washed in 0.1 M phosphate buffer thrice and in double-distilled water repeatedly. All the samples were processed through graded alcohol and then airdried in a vacuum dessicator for 36 hours. The dried samples on coverglass were mounted with carbon glue on polished aluminium stubs, coated with gold of 150 Å thickness in a nanotech evaporator, then photographed and examined under a Cambridge-stereo-Scan S4-10l SEM, operated at 10-20 KV.

On exposure to MOC, the fish, under acute respiratory distress, became inactive and lost their balance. *C. carpio* were affected within 60 minutes and *H. fossilis* survived for 225 minutes. Blood samples were collected just before the fish died.

The SEM observations in control samples showed regularly shaped erythrocytes with the usual smooth surface and central nuclear projections in both species (Figs. 1a, 1b and 1c). On treatment, the blood cells shrunk and became deformed, while poikilocytosis set in (Fig. 1d). At higher magnifications, the cell surface structure showed that the cells were flattened by the liberation of cellular material and bore distinct ruptures all over the surface (Figs. 1e and 1f). This observation pointed to the fact that the MOC treatment led to erythrololysis and ultimate disintegration.

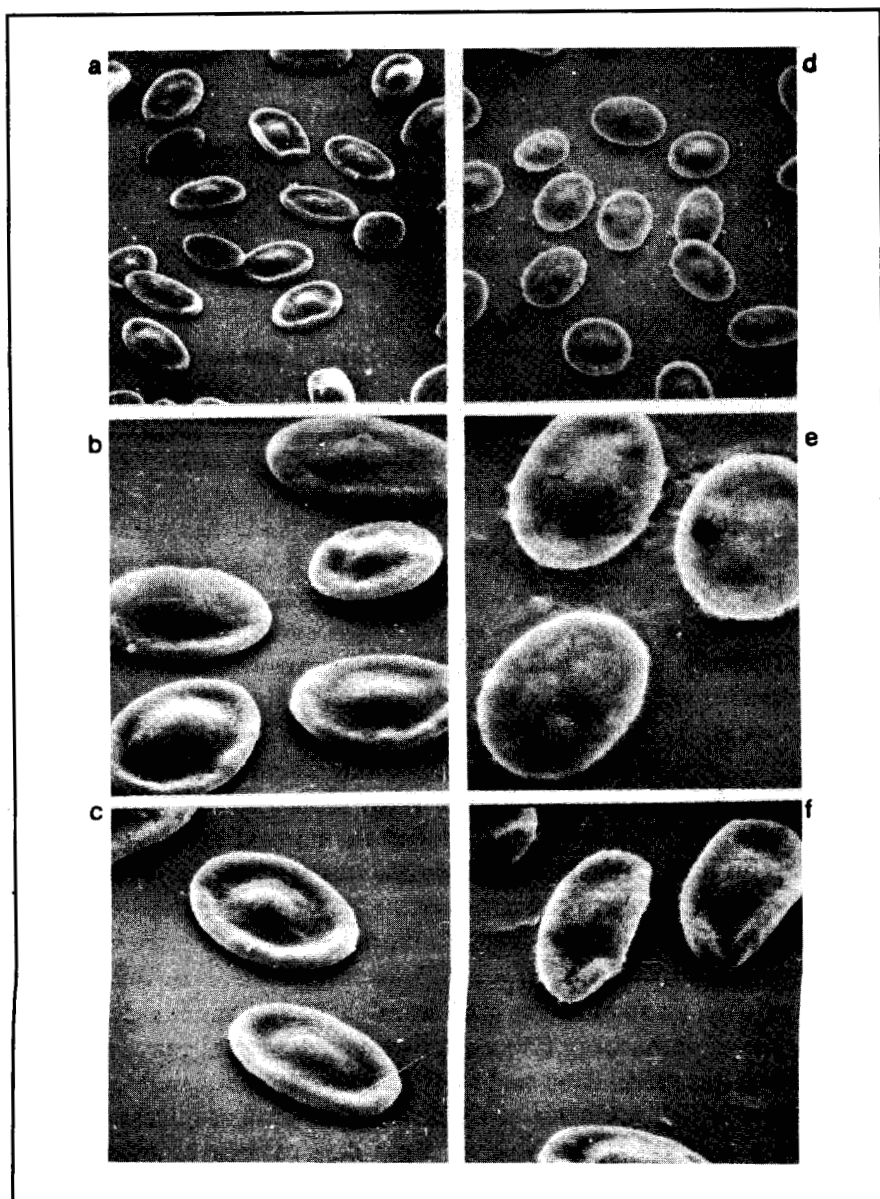


Fig. 1. Scanning electron micrographs of blood cells (a, b, d and e = *Cyprinus carpio*; c and f = *Heteropneustes fossilis*).

- (a, b and c) - Normal erythrocytes with regular shape and smooth surface. (Magnification (a) x 2000, (b and c) x 5000).
- (d, e and f) - Shrunken and flattened RBCs with distinct ruptures in the cell surface on treatment with Mahua oil cake. (Magnification (d) x 2000, (e and f) x 5000).

Generally, red blood cells (RBC) undergo premature destruction by intravascular hemolysis in circulation. This may be caused by trauma to the RBC, either by natural complement activation process to the RBC or by exogenous toxins (Cooper and Bunn 1988). Light microscopic observations (Homechaudhuri et al. 1986) demonstrated severe erythrolysis by saponin dissolved in water and provided a clue to the toxicity of the oil cake.

The present SEM observations confirm earlier results on the erythrolytical action of oil cake. It is hypothesized that saponin, upon entering the blood circulation via the gills and buccal epithelium, causes osmotic tension between erythrocytes and plasma. As hemoglobin moves out through the cell membrane, the RBC gradually shrink and become crenated. There seems to be no such osmotic tension between leukocytes and plasma and hence, the leukocytes remain unaffected by such action (Homechaudhuri 1990). The disintegration of RBCs enhances the possibility of acidosis due to a decrease in the buffering capacity of hemoglobin as well as the loss of available surface area of erythrocytes which play a pivotal role in maintaining the acid-base and ion balance in the blood. The critically decreased oxygen-carrying capacity leads to hypoxia stress, the situation being unsupported by an increase in erythropoietic rate. The resultant symptoms of anemia were distinct in our earlier study (Homechaudhuri et al. 1986).

Cyprinus carpio, being devoid of any accessory respiratory structure, was affected early and suffocated from obstructed gills due to the precipitation of mucus which hindered the normal exchange of gases through the gills. Oxygen tension, due to loss of hemoglobin on rupture of erythrocytes by saponin, further hastened death. On the other hand, *H. fossilis*, with the help of an efficient accessory respiratory structure, overcame gill suffocation.

Probably, as soon as the gills were choked by mucus clogging on stress, the air-breathing fish switched over to aerial respiration. By preventing gill ventilation and further absorption of saponin through the gills, the fish maintained themselves against respiratory stress. However, they could not recover from low oxygen tension, as the hemolytic rate finally surpassed the erythropoietic rate.

Acknowledgements

The authors are indebted to Dr. Hiralal Chaudhuri, University of the Philippines at Los Baños, College, Laguna, Philippines, for his

personal interest in this work and critical suggestions. The technical guidance of Dr. K.K. Misra is also highly appreciated. This work has been supported by a research fellowship sponsored by the University Grants Commission, India.

References

- Bhatia, H.L. 1970. Use of mahua oil cake in fishery management. *Indian Farming* 20: 39-40.
- Chakraborty, P.S., A. Das and S. Banerjee. 1987. Evaluation of efficiency of Mahua oil cake as fish poison in relation to hardness of water. *Environ. Ecol.* 5(1): 165-167.
- CIFRI. 1968. Rep. Central Inland Fish. Res. Inst. Barrackpur, India.
- Cooper, R.A. and A.F. Bunn. 1968. Hemolytic anemias, p. 1,531-1,543. In J.D. Wilson et. al. (eds.), *Principles of Internal Medicine*. McGraw Hill International Book Company.
- Homechaudhuri, S., T. Pandit, S. Poddar and S. Banerjee. 1986. Effect of mahua oil cake on the blood cells and blood values of an air-breathing catfish, *Heteropneustes fossilis* and a carp, *Cyprinus carpio*. *Proc. Indian Acad. Sci. (Anim. Sci.)* 55(5): 617-622.
- Homechaudhuri, S. 1990. Studies on fish haematology in normal condition and under environmental stress. Calcutta University. Ph.D. thesis.
- Lakshmanan, A.K. 1983. Mahua oil cake in fish culture. *Environ. Ecol.* 1: 163-167.